

2002-744US.txt  
 SEQUENCE LISTING

<110> Van der Geize, Robert  
 Hessels, Gerda  
 Dijkhuizen, Lubbert  
 Van der Meijden, Peter

<120> New expression system from Rhodococcus

<130>

<140>

<141>

<150> PCT/EP03/050928

<151> 2003-12-02

<150> EP02080054.6

<151> 2002-12-03

<160> 13

<170> PatentIn Ver. 2.1

<210> 1

<211> 1543

<212> DNA

<213> Rhodococcus erythropolis

<220>

<221> CDS

<222> (1)..(1533)

<400> 1

atg	cag	gac	tgg	acc	agc	gag	tgc	gac	gtg	ttg	gta	gtc	ggc	tcc	ggc	48
Met	Gln	Asp	Trp	Thr	Ser	Glu	Cys	Asp	Val	Leu	Val	Val	Gly	Ser	Gly	
1				5					10					15		

ggc	gga	gcg	ctg	acc	ggc	gca	tat	acc	gcc	gct	gct	cag	gga	ttg	acg	96
Gly	Gly	Ala	Leu	Thr	Gly	Ala	Tyr	Thr	Ala	Ala	Ala	Gln	Gly	Leu	Thr	
			20					25					30			

acg	atc	gtc	ctc	gag	aaa	acc	gat	cgt	ttc	ggc	ggg	acc	tcc	gcc	tac	144
Thr	Ile	Val	Leu	Glu	Lys	Thr	Asp	Arg	Phe	Gly	Gly	Thr	Ser	Ala	Tyr	
		35					40					45				

tcg	ggc	gcc	tcg	atc	tgg	ctc	cca	ggt	acc	cag	gtg	cag	gaa	cgc	gcc	192
Ser	Gly	Ala	Ser	Ile	Trp	Leu	Pro	Gly	Thr	Gln	Val	Gln	Glu	Arg	Ala	
	50					55					60					

gga	ctt	ccc	gac	tcg	acc	gag	aat	gcc	cgc	acc	tat	ctg	cgc	gcg	ttg	240
Gly	Leu	Pro	Asp	Ser	Thr	Glu	Asn	Ala	Arg	Thr	Tyr	Leu	Arg	Ala	Leu	
65					70					75					80	

ctc	ggt	gac	gcc	gag	tcc	gag	cgc	cag	gac	gcc	tac	gtc	gag	acc	gct	288
Leu	Gly	Asp	Ala	Glu	Ser	Glu	Arg	Gln	Asp	Ala	Tyr	Val	Glu	Thr	Ala	
				85					90					95		

ccc	gct	gtc	gtc	gct	cta	ctc	gag	cag	aac	ccg	aac	atc	gaa	ttc	gag	336
Pro	Ala	Val	Val	Ala	Leu	Leu	Glu	Gln	Asn	Pro	Asn	Ile	Glu	Phe	Glu	
			100					105					110			

ttc	cgt	gcg	ttc	ccc	gac	tac	tac	aaa	gcc	gaa	ggc	cgg	atg	gac	acg	384
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

## 2002-744US.txt

Phe	Arg	Ala	Phe	Pro	Asp	Tyr	Tyr	Lys	Ala	Glu	Gly	Arg	Met	Asp	Thr	
		115					120					125				
gga	cgc	tcc	atc	aac	cct	ctc	gat	ctc	gat	ccc	gcc	gac	atc	ggt	gac	432
Gly	Arg	Ser	Ile	Asn	Pro	Leu	Asp	Leu	Asp	Pro	Ala	Asp	Ile	Gly	Asp	
	130					135					140					
ctc	gcc	ggc	aag	gtg	cgt	ccg	gaa	ctg	gac	caa	gac	cgc	acc	ggt	cag	480
Leu	Ala	Gly	Lys	Val	Arg	Pro	Glu	Leu	Asp	Gln	Asp	Arg	Thr	Gly	Gln	
145					150					155					160	
gat	cat	gct	ccc	ggc	ccg	atg	atc	ggt	ggg	cgc	gca	ctg	atc	ggc	cgt	528
Asp	His	Ala	Pro	Gly	Pro	Met	Ile	Gly	Gly	Arg	Ala	Leu	Ile	Gly	Arg	
				165					170					175		
ctg	ctg	gcc	gca	gtt	cag	agc	acc	ggt	aag	gca	gaa	ctt	cgc	acc	gaa	576
Leu	Leu	Ala	Ala	Val	Gln	Ser	Thr	Gly	Lys	Ala	Glu	Leu	Arg	Thr	Glu	
			180					185					190			
tcc	gtc	ctc	acc	tcc	ctg	atc	gtg	gaa	gac	ggc	cgt	gtt	gtc	ggc	gcc	624
Ser	Val	Leu	Thr	Ser	Leu	Ile	Val	Glu	Asp	Gly	Arg	Val	Val	Gly	Ala	
		195					200					205				
gag	gtc	gaa	tcc	ggc	ggc	gaa	acc	cag	cga	atc	aag	gcg	aac	cgc	ggt	672
Glu	Val	Glu	Ser	Gly	Gly	Glu	Thr	Gln	Arg	Ile	Lys	Ala	Asn	Arg	Gly	
	210					215					220					
gtc	ctg	atg	gca	gca	ggc	ggc	atc	gaa	ggc	aac	gcc	gag	atg	cgt	gag	720
Val	Leu	Met	Ala	Ala	Gly	Gly	Ile	Glu	Gly	Asn	Ala	Glu	Met	Arg	Glu	
225					230					235					240	
cag	gca	ggc	acc	ccc	ggc	aag	gcg	atc	tgg	agt	atg	ggt	ccc	ttc	ggc	768
Gln	Ala	Gly	Thr	Pro	Gly	Lys	Ala	Ile	Trp	Ser	Met	Gly	Pro	Phe	Gly	
				245					250					255		
gcc	aac	acc	ggc	gac	gcg	atc	tct	gcc	ggt	att	gct	gtc	ggc	ggc	gca	816
Ala	Asn	Thr	Gly	Asp	Ala	Ile	Ser	Ala	Gly	Ile	Ala	Val	Gly	Gly	Ala	
			260					265					270			
aca	gcc	ttg	ctc	gat	cag	gcg	tgg	ttc	tgc	ccc	ggc	gtc	gag	cag	ccc	864
Thr	Ala	Leu	Leu	Asp	Gln	Ala	Trp	Phe	Cys	Pro	Gly	Val	Glu	Gln	Pro	
		275					280					285				
gac	ggc	agc	gcc	gcc	ttc	atg	gtc	ggc	gtt	cgc	ggt	ggg	ctc	gtc	gtc	912
Asp	Gly	Ser	Ala	Ala	Phe	Met	Val	Gly	Val	Arg	Gly	Gly	Leu	Val	Val	
	290					295					300					
gac	agc	gcc	ggt	gag	cgc	tac	ctc	aac	gag	tcg	ctt	ccg	tac	gac	cag	960
Asp	Ser	Ala	Gly	Glu	Arg	Tyr	Leu	Asn	Glu	Ser	Leu	Pro	Tyr	Asp	Gln	
305					310					315					320	
ttc	gga	cga	gcc	atg	gat	gct	cac	gac	gac	aac	ggt	tct	gcc	gtg	ccg	1008
Phe	Gly	Arg	Ala	Met	Asp	Ala	His	Asp	Asp	Asn	Gly	Ser	Ala	Val	Pro	
				325					330					335		
tcg	ttc	atg	atc	ttc	gac	tcg	cgc	gag	ggt	ggc	gga	ctg	ccc	gcc	atc	1056
Ser	Phe	Met	Ile	Phe	Asp	Ser	Arg	Glu	Gly	Gly	Gly	Leu	Pro	Ala	Ile	
			340					345					350			
tgc	atc	ccg	aac	acg	gcg	ccc	gcc	aag	cac	ctc	gaa	gcc	gga	acg	tgg	1104
Cys	Ile	Pro	Asn	Thr	Ala	Pro	Ala	Lys	His	Leu	Glu	Ala	Gly	Thr	Trp	
		355					360					365				

2002-744US.txt

gtc ggt gcc gac act ctc gaa gaa ctc gct gcc aag acc gga cta ccg Val Gly Ala Asp Thr Leu Glu Glu Leu Ala Lys Thr Gly Leu Pro	1152
370 375 380	
gcc gac gca ttg cgc agc act gtc gaa aag ttc aac gat gcc gca aaa Ala Asp Ala Leu Arg Ser Thr Val Glu Lys Phe Asn Asp Ala Ala Lys	1200
385 390 395 400	
ctg ggc gtc gac gaa gag ttc cat cgc ggc gaa gac ccg tac gac gcg Leu Gly Val Asp Glu Glu Phe His Arg Gly Glu Asp Pro Tyr Asp Ala	1248
405 410 415	
ttc ttc tgc cca ccc aac ggc ggt gcg aat gcg gca ctg acg gcc atc Phe Phe Cys Pro Pro Asn Gly Gly Ala Asn Ala Ala Leu Thr Ala Ile	1296
420 425 430	
gag aac gga ccg ttc tac gcg gcc cgc atc gtc ctc agt gac ctc ggc Glu Asn Gly Pro Phe Tyr Ala Ala Arg Ile Val Leu Ser Asp Leu Gly	1344
435 440 445	
acc aag ggc gga ttg gtc acc gac gtc aac ggc cga gtc ctg cgt gct Thr Lys Gly Gly Leu Val Thr Asp Val Asn Gly Arg Val Leu Arg Ala	1392
450 455 460	
gac ggc agc gcc atc gac ggc ctg tac gcc gcc ggc aac acg agc gcg Asp Gly Ser Ala Ile Asp Gly Leu Tyr Ala Ala Gly Asn Thr Ser Ala	1440
465 470 475 480	
tca ctg agc ggc cgc ttc tac ccc ggc ccc gga gtt cca ctc ggc acg Ser Leu Ser Gly Arg Phe Tyr Pro Gly Pro Gly Val Pro Leu Gly Thr	1488
485 490 495	
gct atg gtc ttc tcg tac cga gca gct cag gac atg gcg aag taa Ala Met Val Phe Ser Tyr Arg Ala Ala Gln Asp Met Ala Lys	1533
500 505 510	
cgcagttcaa	1543

<210> 2  
 <211> 511  
 <212> PRT  
 <213> Rhodococcus erythropolis

<400> 2  
 Met Gln Asp Trp Thr Ser Glu Cys Asp Val Leu Val Val Gly Ser Gly  
 1 5 10 15  
 Gly Gly Ala Leu Thr Gly Ala Tyr Thr Ala Ala Ala Gln Gly Leu Thr  
 20 25 30  
 Thr Ile Val Leu Glu Lys Thr Asp Arg Phe Gly Gly Thr Ser Ala Tyr  
 35 40 45  
 Ser Gly Ala Ser Ile Trp Leu Pro Gly Thr Gln Val Gln Glu Arg Ala  
 50 55 60  
 Gly Leu Pro Asp Ser Thr Glu Asn Ala Arg Thr Tyr Leu Arg Ala Leu  
 65 70 75 80  
 Leu Gly Asp Ala Glu Ser Glu Arg Gln Asp Ala Tyr Val Glu Thr Ala  
 85 90 95

## 2002-744US.txt

Pro Ala Val Val Ala Leu Leu Glu Gln Asn Pro Asn Ile Glu Phe Glu  
 100 105 110  
 Phe Arg Ala Phe Pro Asp Tyr Tyr Lys Ala Glu Gly Arg Met Asp Thr  
 115 120 125  
 Gly Arg Ser Ile Asn Pro Leu Asp Leu Asp Pro Ala Asp Ile Gly Asp  
 130 135 140  
 Leu Ala Gly Lys Val Arg Pro Glu Leu Asp Gln Asp Arg Thr Gly Gln  
 145 150 155 160  
 Asp His Ala Pro Gly Pro Met Ile Gly Gly Arg Ala Leu Ile Gly Arg  
 165 170 175  
 Leu Leu Ala Ala Val Gln Ser Thr Gly Lys Ala Glu Leu Arg Thr Glu  
 180 185 190  
 Ser Val Leu Thr Ser Leu Ile Val Glu Asp Gly Arg Val Val Gly Ala  
 195 200 205  
 Glu Val Glu Ser Gly Gly Glu Thr Gln Arg Ile Lys Ala Asn Arg Gly  
 210 215 220  
 Val Leu Met Ala Ala Gly Gly Ile Glu Gly Asn Ala Glu Met Arg Glu  
 225 230 235 240  
 Gln Ala Gly Thr Pro Gly Lys Ala Ile Trp Ser Met Gly Pro Phe Gly  
 245 250 255  
 Ala Asn Thr Gly Asp Ala Ile Ser Ala Gly Ile Ala Val Gly Gly Ala  
 260 265 270  
 Thr Ala Leu Leu Asp Gln Ala Trp Phe Cys Pro Gly Val Glu Gln Pro  
 275 280 285  
 Asp Gly Ser Ala Ala Phe Met Val Gly Val Arg Gly Gly Leu Val Val  
 290 295 300  
 Asp Ser Ala Gly Glu Arg Tyr Leu Asn Glu Ser Leu Pro Tyr Asp Gln  
 305 310 315 320  
 Phe Gly Arg Ala Met Asp Ala His Asp Asp Asn Gly Ser Ala Val Pro  
 325 330 335  
 Ser Phe Met Ile Phe Asp Ser Arg Glu Gly Gly Gly Leu Pro Ala Ile  
 340 345 350  
 Cys Ile Pro Asn Thr Ala Pro Ala Lys His Leu Glu Ala Gly Thr Trp  
 355 360 365  
 Val Gly Ala Asp Thr Leu Glu Glu Leu Ala Ala Lys Thr Gly Leu Pro  
 370 375 380  
 Ala Asp Ala Leu Arg Ser Thr Val Glu Lys Phe Asn Asp Ala Ala Lys  
 385 390 395 400  
 Leu Gly Val Asp Glu Glu Phe His Arg Gly Glu Asp Pro Tyr Asp Ala  
 405 410 415  
 Phe Phe Cys Pro Pro Asn Gly Gly Ala Asn Ala Ala Leu Thr Ala Ile  
 420 425 430

2002-744US.txt

Glu Asn Gly Pro Phe Tyr Ala Ala Arg Ile Val Leu Ser Asp Leu Gly  
 435 440 445  
 Thr Lys Gly Gly Leu Val Thr Asp Val Asn Gly Arg Val Leu Arg Ala  
 450 455 460  
 Asp Gly Ser Ala Ile Asp Gly Leu Tyr Ala Ala Gly Asn Thr Ser Ala  
 465 470 475 480  
 Ser Leu Ser Gly Arg Phe Tyr Pro Gly Pro Gly Val Pro Leu Gly Thr  
 485 490 495  
 Ala Met Val Phe Ser Tyr Arg Ala Ala Gln Asp Met Ala Lys  
 500 505 510

<210> 3  
 <211> 158  
 <212> DNA  
 <213> Rhodococcus erythropolis

<400> 3  
 atcatcgatt atgtgtcccg gccgcgaacg accgcgctaa ttctctcacc tggaccaccc 60  
 atctcggcat attgcccgt cagtgggacc tggcatggcc ttccagtgcc gtgcggtatt 120  
 ccgtggacac cccaccctct tggagtaagg acgcaatg 158

<210> 4  
 <211> 19  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:primer

<400> 4  
 ggcgacgttg ccgagaatt 19

<210> 5  
 <211> 624  
 <212> DNA  
 <213> Rhodococcus erythropolis

<220>  
 <221> CDS  
 <222> (1)..(624)

<400> 5  
 atg ggg gcg acg ttg ccg aga att gcc gag gtc agg gac gct gct gag 48  
 Met Gly Ala Thr Leu Pro Arg Ile Ala Glu Val Arg Asp Ala Ala Glu  
 1 5 10 15  
 ccc agt tcg gac gag cag cgg gcg cgc cat gtg cgg atg ctg gaa gcg 96  
 Pro Ser Ser Asp Glu Gln Arg Ala Arg His Val Arg Met Leu Glu Ala  
 20 25 30  
 gcc gcc gaa ttg ggg acc gag aaa gaa ctc tca cgg gtt cag atg cac 144  
 Ala Ala Glu Leu Gly Thr Glu Lys Glu Leu Ser Arg Val Gln Met His  
 35 40 45

## 2002-744us.txt

gaa gtt gcc aag cgg gca ggc gtg gcc atc ggc act ctc tac cgc tat 192  
 Glu Val Ala Lys Arg Ala Gly Val Ala Ile Gly Thr Leu Tyr Arg Tyr  
 50 55 60

ttc cct tcg aag acg cac ctc ttc gtc gct gtg atg gtc gag cag atc 240  
 Phe Pro Ser Lys Thr His Leu Phe Val Ala Val Met Val Glu Gln Ile  
 65 70 75 80

gat cag atc ggc gac agt ttc gcc aag cat cag gtg cag tcg gcc aat 288  
 Asp Gln Ile Gly Asp Ser Phe Ala Lys His Gln Val Gln Ser Ala Asn  
 85 90 95

ccg cag gac gcc gtg tac gag gtc ctg gtg cgc gcg act cgc ggg tta 336  
 Pro Gln Asp Ala Val Tyr Glu Val Leu Val Arg Ala Thr Arg Gly Leu  
 100 105 110

ctg cgt cgg ccg gcc ctt tcg act gcg atg ctg cag tcg tcc agt acc 384  
 Leu Arg Arg Pro Ala Leu Ser Thr Ala Met Leu Gln Ser Ser Ser Thr  
 115 120 125

gcc aac gtc gcg acg gtg ccg gac gtg ggc aag atc gat cgc ggc ttc 432  
 Ala Asn Val Ala Thr Val Pro Asp Val Gly Lys Ile Asp Arg Gly Phe  
 130 135 140

cgg cag atc atc ctc gat gcg gcc ggg atc gag aac ccg acc gag gaa 480  
 Arg Gln Ile Ile Leu Asp Ala Ala Gly Ile Glu Asn Pro Thr Glu Glu  
 145 150 155 160

gac aac acc ggg ttg cgt ctg ctg atg cag ctg tgg ttc ggg gtc atc 528  
 Asp Asn Thr Gly Leu Arg Leu Leu Met Gln Leu Trp Phe Gly Val Ile  
 165 170 175

caa tcg tgc ctc aac ggt cga att tcc atc ccg gat gcg gag tac gac 576  
 Gln Ser Cys Leu Asn Gly Arg Ile Ser Ile Pro Asp Ala Glu Tyr Asp  
 180 185 190

atc cgc aag ggg tgc gac ctg ctt ctg gtg aat ctc tca cga cac tga 624  
 Ile Arg Lys Gly Cys Asp Leu Leu Leu Val Asn Leu Ser Arg His  
 195 200 205

&lt;210&gt; 6

&lt;211&gt; 208

&lt;212&gt; PRT

&lt;213&gt; Rhodococcus erythropolis

&lt;400&gt; 6

Met Gly Ala Thr Leu Pro Arg Ile Ala Glu Val Arg Asp Ala Ala Glu  
 1 5 10 15

Pro Ser Ser Asp Glu Gln Arg Ala Arg His Val Arg Met Leu Glu Ala  
 20 25 30

Ala Ala Glu Leu Gly Thr Glu Lys Glu Leu Ser Arg Val Gln Met His  
 35 40 45

Glu Val Ala Lys Arg Ala Gly Val Ala Ile Gly Thr Leu Tyr Arg Tyr  
 50 55 60

Phe Pro Ser Lys Thr His Leu Phe Val Ala Val Met Val Glu Gln Ile  
 65 70 75 80

Asp Gln Ile Gly Asp Ser Phe Ala Lys His Gln Val Gln Ser Ala Asn

85

95

Pro Gln Asp Ala Val Tyr Glu Val Leu Val Arg Ala Thr Arg Gly Leu  
100 105 110  
Leu Arg Arg Pro Ala Leu Ser Thr Ala Met Leu Gln Ser Ser Ser Thr  
115 120 125  
Ala Asn Val Ala Thr Val Pro Asp Val Gly Lys Ile Asp Arg Gly Phe  
130 135 140  
Arg Gln Ile Ile Leu Asp Ala Ala Gly Ile Glu Asn Pro Thr Glu Glu  
145 150 155 160  
Asp Asn Thr Gly Leu Arg Leu Leu Met Gln Leu Trp Phe Gly Val Ile  
165 170 175  
Gln Ser Cys Leu Asn Gly Arg Ile Ser Ile Pro Asp Ala Glu Tyr Asp  
180 185 190  
Ile Arg Lys Gly Cys Asp Leu Leu Leu Val Asn Leu Ser Arg His  
195 200 205

<210> 7  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:primer

<400> 7  
tcagtgtcgt gagagattca

20

<210> 8  
<211> 35  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:primer

<400> 8  
ataaagctta tcgattatgt gtcccggccg cgaac

35

<210> 9  
<211> 35  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:primer

<400> 9  
ataggtacca tatgtgcgtc cttactccaa gaggg

35

<210> 10  
<211> 27  
<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:primer

<400> 10

gcgcatatgg ctaagaatca ggcaccc

27

<210> 11

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:primer

<400> 11

gcgggatccc tacttctctg ctgcgtgatg

30

<210> 12

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:primer

<400> 12

ggccatatgt tgaccacaga cgtgacgacc

30

<210> 13

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:primer

<400> 13

gccactagtt cactgcgctg ctctgcacg

30